CONTRIBUTION TO THE STUDIES OF COAGULOGRAM IN THOROUGHBRED HORSES *

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Referring to the literature on the subject, we see a few controversial points have been established in relation to the coagulation phenomenon in horses. Soulier and Larrieu (55) suggested that horses' blood had the coagulation factors on concentration similar to that of human beings but Bell et al. (11 and 12) came to the conclusion that notable difference exist between these factors of the two mentioned species believing that horses' blood is deficient in antihemophilic globulin. Sjolin (52), disagreeing with the above writers, first admitted deficiency of Christmas factor but afterwards (53) set a different concept then accepting that there was probaly an insufficiency of a factor quite similar or identical to Hageman's. Barkhan et al. (7) acquiesced with the works of Bell et al. (11 and 12) while Fantl and Marr (25), on the other side, concluded that the thromboplastinic factors of the blood of human beings and horses markedly differ in quantity but are quite similar in their action. Grecchi et al. (29), ratified Bell's et al. (12) previous conclusion about the differences in thromboplastinic action.

Fantl and Marr (25) found out that it occurs greater activity of Factor V in horses' blood than in human blood, although Barkhan et al. (7) noticed no differences between them in the whole prothrombinic complex.

Fantl and Ward (26) demonstrated that quantity of prothrombine in horses's normal blood is similar to that in man's thrombocitopenic blood, which suits the affirmation that animal has inadequate number of platelets thus presenting smaller tendency to free phospholipids (Fantl and Marr — 25).

Spontaneous hemorrhages rarely occur in horses (Miller — 37) whereas these animals are entitled to have hemophilioid diseases or others related to the processes

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of coagulation or hemostasis in horses. Barkhan (6) calls the attention to the question even though he did not yet observe any real case of it. The petequial fever, morbo maculoso or hemorrhagic purpura in horses, nosologic entity known for a long time, having a discussed etiology would be a non-thrombocitopenic purpura, as concluded by Biggers et al. (13). Hemorrhagic states after castration, has been object of study by several authors (19, 49, 64) and it has been denominated by Chapron (18), Castration Hemophily, who admitted that hormonal disturbances should probably be the cause for it. Volkmar (60), also described hemorrhagic diseases in various animals including horses, in which coagulation time was too long or at times blood coagulation didn't even occur.

As to horses' epistaxis, whose origin is quite unknown, is not yet completely set it to be independent of alterations in blood coagulation, as well as the infeccious anemies in horses, which may alter the coagulation process (31 and 33).

Several authors (17, 18, 22, 24, 34, 40, 42, 57) have done qualitative studies comparing blood coagulation in horses and other species.

Experiments for avaliation of the blood coagulation phenomenon and determination of normal values for horses, were subject of a few written works which, however, due to several reasons, cannot be used as standards for the whole specie either because tests have been made with few or not accurately characterized animals or because they were not perfectly padronized. Concerning literature, as follows: Adams (1), Aparici (2), Archer (4), Awad and Morcos (5), Barkhan (7), Behrens (9), Bell et al. (12), Burker (16), Diaz (23), Florio (28), Schwayer (50), Sippel (51), Sopeña (54), Van Wassenhove (58), Villard (59), Weiser (62).

So, in order to clarify facts which would permit to precise the true character of the interrelated factors in blood coagulation in horses, as well as to set the real scheme of the different hemorrhagic syndromes in this whole specie, it seemed to us fundamentally important to determine the normal values for the commonest tests used in the avaliation of blood coagulation. Thoroughbred Horses were chosen for this work because they have great clinic interest, they are a quite homogeneous group and they offer great possibilities for investigation of hereditary factors if any pathological process appears.

Contradictory notes can be found about the hormonal influence on blood coagulation (8, 21, 32, 36). So, in our experiment we aimed at the determination of the average populational values for the tests of: coagulation time, coagulation time of recalcified plasma, platelet counts, clot retraction, prothrombin time, thromboplastin generation test, at the same time that we tried to prove the nullity hypothesis of non-difference between sex, in relation to the mentioned tests, adopting the rejection level of 5%.

Material and methods

The blood submitted to different experiments at the Jockey Club of S. Paulo, was taken out of fifty Thoroughbred horses 25 females and 25 male horses between 2 and 5 years, considered healthy clinical, neither presenting serious nor slight hemorrhages and all of them were submitted to a similar diet. The material was collected from the jugular vein, being a siliconized syringe used after the animals rest, this is, three hours after the morning training.

Coagulation time — It was performed by according to Lee White's technique (35), using two tubes of 100/13 mm and the reading was done every 60 seconds.

Coagulation time of recalcified plasma — The technique described in Quick (44 — pg. 363) was adopted. We obtained the plasma used by centrifugation at 1.500 r.p.m. during 10 minutes of oxalated blood at 10% with an acqueous solution of 0.1 M of sodium oxalate. The test was made within the first hour after the blood was taken out and the calcium used came from a 0.02 M solution of calcium chloride. The test performed in two 100/13 mm tubes had its result expressed by the tube that coagulated in the first place.

Platelet counts — The blood for the platelet counts was collected in siliconized flasks containing the dissodic salt of the ethylenediaminotetracetic acid (EDTA Na_2) in a 10% solution and in the proportion of 5 mg of salt for 5 ml of blood. The method of Feissly and Lndin (27) modified by Rosenfeld (56) was followed. The suspension was made in a hematimetric pipette for leucocyte counts, in the proportion of 1:20 and the count in the Neubauer camera under phase microscopy, in a 2 mm^2 area.

Clot retraction — We measured according to the method preconized by Rosenfeld (46). The test was performed in a water-bath at the temperature of 37°C and the reading made three hours after the coagulation.

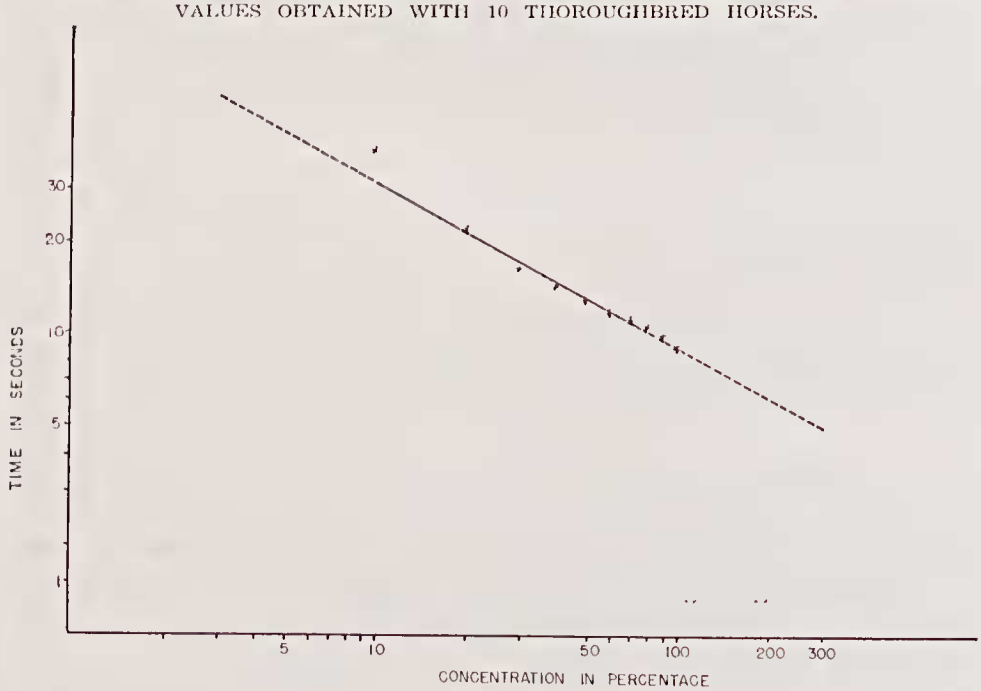
Prothrombin time (prothrombin complex) — The method of a stage introduced by Quick (39) was followed in the performance of this test we used the same oxalated plasma utilized for the coagulation time of recalcified plasma test. The thromboplastin was prepared with the rabbit's brain according to Quick's technique (41). The thromboplastin suspensions were preserved in the ice box +5°C and used in the maximum for 10 days, considered adequate for the use when the

time of normal prothrombin, this is, 11 to 12 seconds was given to the human plasma. The calcium chloride used was 0.02 M. The verifications were made three times and it was considered as the result which was repeated; when there was a disagreement, new determinations were made until the values agreed. Curves of prothrombin dilution were made for the transformation of the data in concentration.

In order to perform this curve, plasma of 10 animals was obtained, which presented a prothrombin time near to the medium value. Two series of dilutions were prepared for each animal and the average of the values obtained in the first and second were calculated. The average of each concentration in the 10 animals represented the normal index for that concentration. The same criterion described for the reading of prothrombin time is here followed. The final curve transcribed to the dilogarithmic paper, can be changed, permitting the extrapolation of the values not within the amplitude of the data (graph. 1). The results expressed % of time of the prothrombinic complex.

FIGURE I

REFERENCE CURVE OF PROTHROMBINIC ACTIVITY IN RELATION TO TIME IN SECONDS AND PERCENTAGE OF CONCENTRATION, CONSTRUCTED WITH VALUES OBTAINED WITH 10 THOROUGHBRED HORSES.



Consumption of prothrombin — The technique of Quick (43) was used modified by Rosenfeld (48). 5 ml of blood were taken out placed in tubes of 100/13 mm and left in a water-bath at a temperature of 37°C. When it coagulated, the time was marked and the displacement of the clot was promoted. 59 minutes after the coagulation, the material was centrifuged for one minute and the supernatant was taken off and afterwards kept, in ice-bath; the quantity of residual prothrombin was tested by the time it took to coagulate the mixture of this serum with thromboplastin and calcium, having as a resource of fibrinogenous oxalated plasma absorbed by barium sulphate, washed according to the technique described by Biggs and MacFarlane (15) used in the proportion of 0.1 g for 1 ml of plasma. The absorption took place in the water-bath for 30 minutes; the tubes were agited from time to time and afterwards they were centrifuged at 2,500 r.p.m. during 10 minutes, being the supernatant taken off. The plasma was considered adequate for use, when its prothrombin time was more than 4 minutes. As a resource of calcium, the 0.02 M solution of calcium chloride was also adequate. Three tests were performed, that followed the same criterion adopted for the reading of the prothrombin time. The times obtained were transformed in concentration by means of the curve of prothrombin dilution, and that represented the residual prothrombin after the evolution of the coagulation process for 60 minutes. Knowing the percentual quantity of the plasma prothrombin, the prothrombin was obtained — consumed by the following calculations:

$$C = \frac{100 \times B}{A}$$

$$D = 100 - C$$

A = Total prothrombin expressed in % of time.

B = Residual prothrombin expressed in %.

C = Residual concentration calculated at a rate of 100% of total prothrombin for the animal.

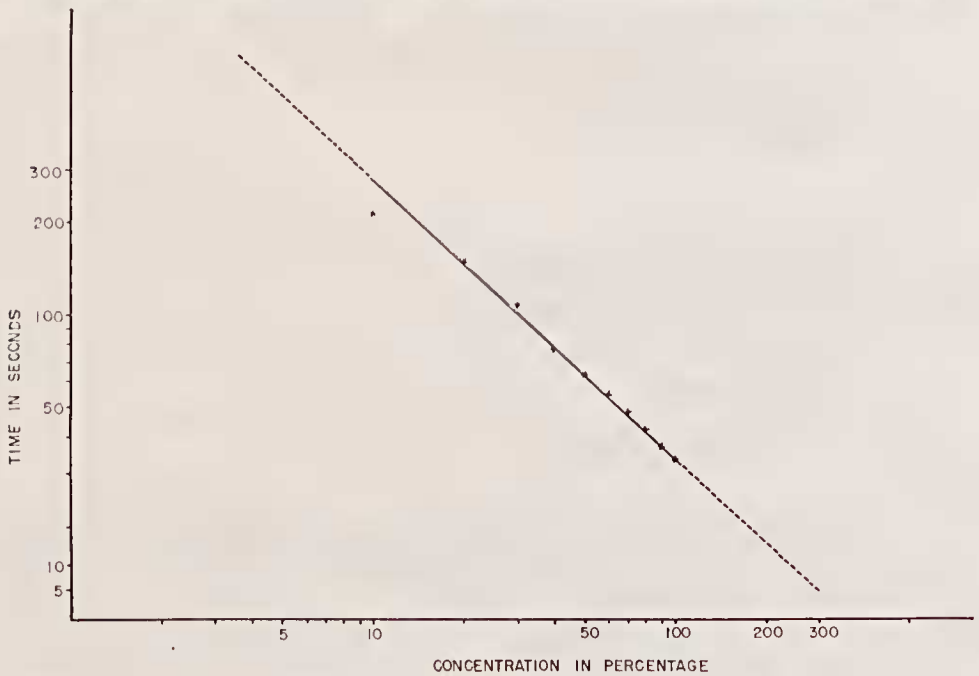
D = % of consumed prothrombin.

Thromboplastin generation — Biggs and Douglas's (14) technique was followed. The animals were divided in 2 groups of 24 and 26, formed of male and female horses in equal numbers. In the first group, the test was performed with suspension of the platelets of the own animal and in the second, the phospho-

lipids obtained of human cerebrum treated by acetone, according to Bell's and Alton's (10) technique. The citrated plasma absorbed by the oxalated plasma treated with sulphate of barium, according to the rule above described. The platelets for suspension came from the blood collected with sodium citrate in a physiological solution at 3.8% in the proportion of 10% of the total volume. The platelets were washed twice in a solution containing 3.8 g of sodium citrate and 0.1 g of dissodic salt of the ethylenediaminotetracetic acid for 100 ml of distilled water and afterwards washed once more with a physiological solution, being finally suspended in the same solution obeying a concentration, that is about three times bigger than be one of the plasma. In the systems prepared with phospholipids in substitution to the suspension of platelets, a preparation was used that contained 0.12 mg of that material by ml, the most active concentration of a tested series. The resource of fibrinogen was plasma oxalated from the own animal, poor in platelets and of calcium, calcium chloride in a 0.2 M solution. The test lated 8 minutes; two verifications were made for each animal and the

FIGURE II

CURVE OF THROMBOPLASTINIC ACTIVITY WITH PLATELETS SYSTEM IN RELATION TO TIME IN SECONDS AND PERCENTAGE OF CONCENTRATION, CONSTRUCTED WITH VALUES OBTAINED WITH 5 THOROUGHBRED HORSES.



result corresponded to the average of these tests. A thromboplastin dilution curve for the transformation of the results in concentrations was built. For this, 5 Thoroughbred horses were used, chosen among a many others, which presented in the thromboplastin generation, an index of high activity. For every animal, 2 series of dilutions with platelets were prepared and 2 with cephaline, from systems in which the generation was interrupted by a sudden fall of the temperature in ice-bath, when the generation was at the maximum and considered 100%. Three determinations of activity for each concentration were done the average was calculated. For every animal, one dilution curve for the platelets and another for the cephaline was obtained, given by the average of every concentration in the two dilution series. The average of the curves in the 5 animals tested, provided the standard-values, which could be checked on the dilogarithmic paper, permitting the extrapolation of the values, not within the amplitude of the data (graphs II and III).

FIGURE III

REFERENCE CURVE OF THROMBOPLASTINIC ACTIVITY WITH PHOSPHOLIPID SYSTEM IN SECONDS AND PERCENTAGE OF CONCENTRATION, CONSTRUCTED WITH VALUES OBTAINED WITH 5 THOROUGHBRED HORSES.

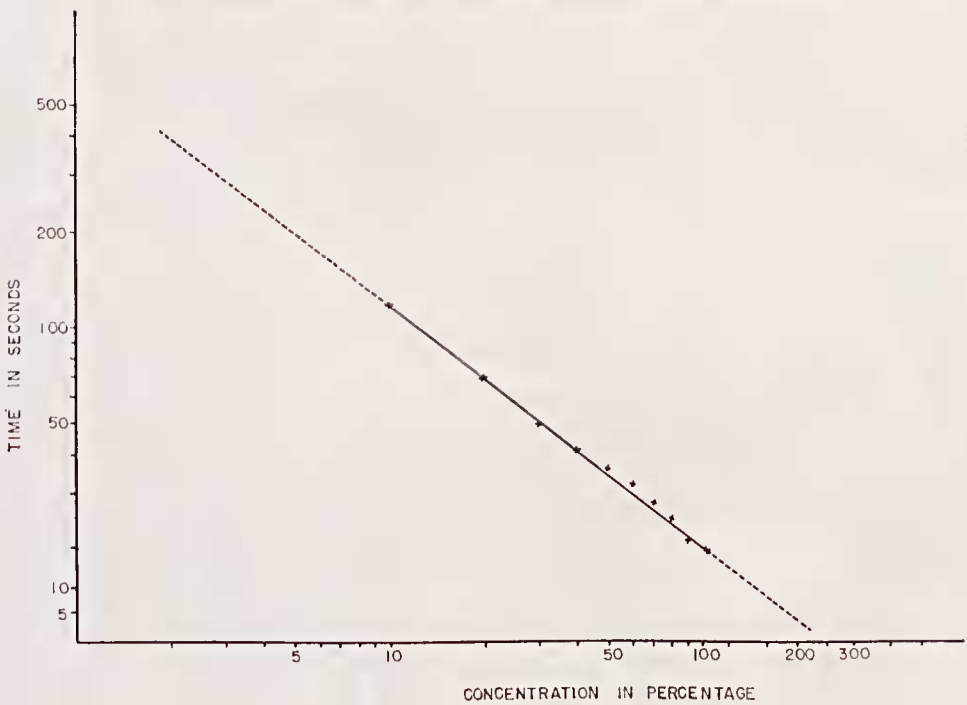


TABLE I — SUMMARIZED DATA OBTAINED FOR THOROUGHBRED HORSES, ACCORDING TO SEX, IN RELATION TO THE TESTS PERFORMED AND TO THE CALCULATED MEASURES OF POSITION AND VARIABILITY

Tests / Measures	Clotting time (minutes)				Clotting time of recalcified plasma (sec.)		Platelets 10^3 mm^3		Clot retraction		Prothrombin time (sec.)		Prothrombin consumption (%)	
	1st tube		2nd tube											
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Range	6,0 to 11,0	6,0 to 9,0	7,0 to 12,0	6,0 to 12,0	90,0 to 420,0	120,0 to 360,0	50,0 to 190,0	50,0 to 170,0	26,0 to 58,0	33,0 to 54,0	5,0 to 13,0	5,0 to 14,0	50,0 to 100,0	50,0 to 95,0
Mean	7,8	7,1	9,3	8,4	244,8	236,4	106,0	104,0	41,8	44,9	8,8	8,4	77,2	79,8
Standard deviation	1,1	1,1	1,4	1,2	73,3	64,8	37,3	29,0	6,6	5,0	2,1	2,9	14,4	12,6
Median	8,0	7,0	9,0	8,0	240,0	240,0	100,0	110,0	42,0	46,0	9,0	8,0	80,0	80,0
Pearson's coefficient of variability (%)	14,3	15,4	15,6	14,2	29,9	27,4	37,3	27,8	15,8	11,2	23,8	34,5	18,7	15,7
95% confidence interval for mean	7,3 to 8,2	6,6 to 7,5	8,6 to 9,9	7,8 to 8,9	213,8 to 275,7	209,0 to 263,7	90,2 to 121,7	91,7 to 116,2	38,9 to 43,4	42,7 to 47,0	7,9 to 9,6	7,1 to 9,6	71,1 to 83,2	74,4 to 85,1

TABLE II — SUMMARIZED DATA OBTAINED FOR THOROUGHBRED HORSES, ACCORDING TO SEX, IN RELATION TO THE THROMBOPLASTIN GENERATION TEST AND THE MEASURES OF POSITION AND VARIABILITY

Measures \ Tests	THROMBOPLASTIN GENERATION									
	Systems with cephalin					Systems with platelets				
	Maximum generation (min.)		Maximum activity			Maximum generation (min.)		Maximum activity		
			In clotting time (sec.)		In concentration (%)			In clotting time (sec.)		In concentration (%)
	M	F	M	F		M	F	M	F	
Range	2,0 to 5,0	2,0 to 5,0	14,0 to 37,0	12,5 to 34,0	45,0 to 131,0	2,0 to 5,0	2,0 to 5,0	18,0 to 55,0	20,5 to 60,0	53,0 to 185,5
Mean	3,3	3,0	18,1	18,0	107,2	3,3	3,5	35,0	36,7	101,0
Standard deviation	1,0	0,9	6,7	5,5	20,5	0,7	0,9	9,7	11,2	31,7
Median	3,0	3,0	16,0	17,0	110,0	3,0	3,0	35,5	38,7	96,2
Pearson's coefficient of variability (%)	31,2	30,3	37,9	30,4	19,1	23,3	28,2	27,8	30,5	31,4
95% confidence interval for mean	2,6 to 3,9	2,4 to 3,5	13,8 to 22,3	14,6 to 21,5	98,7 to 115,7	2,7 to 3,8	2,8 to 4,1	28,5 to 41,5	29,2 to 44,2	87,3 to 114,7

M = male
F = female

TABLE III — SUMMARIZED DATA OBTAINED FOR THOROUGHBRED HORSES IN RELATION TO THE TESTS PERFORMED AND THE CALCULATED MEASURES OF POSITION AND VARIABILITY

Tests / Measures	Clotting time (minutes)		Clotting time of recalcified plasma (sec.)	Platelet (10^3/mm^3)	Clot retraction (%)	Prothrombin time concentration		Prothrombin consumption (%)	Thromboplastin generation					
									Cephalin			Platelets		
	1st tube	2nd tube				(sec.)	(%)		Max. gener. min.	Max. activ. in C.T. (sec.)	Max. activ. in concent. (%)	Max. gener. min.	Max. activ. in C.T. (sec.)	Max. activ. in concent. (%)
Range	6	6	90	50	26	5	50	50	2	12,5	45	2	18	53
	to		to	to	to	to	to	to	to	to	to	to	to	to
	11	12	420	180	58	14	290	100	5	37,0	131	5	60	185
Mean	7,5	8,9	240,6	105,6	43,3	8,6	120,5	78,5	3,1	18,0	107,2	3,4	35,9	101,0
Standard deviation	1,1	1,4	69,2	33,0	6,0	2,5	58,0	13,5	0,9	6,0	20,5	0,8	10,3	31,7
Median	7,0	9,0	240,0	100,0	43,0	8,0	120,0	80,0	3,0	17,0	110,0	3,0	34,5	96,2
Pearson's coefficient of variability (%)	15,2	16,0	28,5	31,5	13,9	29,5	48,1	17,2	30,9	33,4	19,1	25,8	28,7	31,4
	7,1	8,5	221,4	95,9	41,7	7,9	104,1	74,7	2,7	15,6	98,7	3,0	31,4	87,3
95% confidence interval for mean	to	to	to		to	to	to	to	to	to	to	to	to	to
	7,8	9,2	259,7	114,1	45,0	9,3	136,5	82,2	3,5	20,5	115,7	3,7	40,3	114,7

Results

The average values, the standard deviation, the median, Pearson's coefficient of variability, as well as the estimations of the confidence interval of 95% for the average was calculated for male and female horses, in the different tests performed. These same values were calculated, according to the grouped data of male and female horses.

In table I, we can find the results obtained in the tests of coagulation time, coagulation time of recalcified plasma, platelet count, clot retraction, prothrombin time and prothrombin consumption for male and female horses. In table II, there are the results of the tests of thromboplastin generation, concerning the minute when the maximum generation occurred and the maximum activity was observed in male and female horses, expressed by the time it took to coagulate the plasma of the animal, poor in platelets plus calcium.

The results obtained in all the tests performed, calculated according to the grouped data of male and female horses, are summarized on table III, while on table IV, we have the summary of the values found in the literature for normal equines, according to the different authors, including our discoveries.

Graph IV and V illustrate the distribution of frequency of the P.S.I. equines in function of the time in which the thromboplastin generation in systems with platelets and phospholipids, respectively, was maximum.

FIGURE V

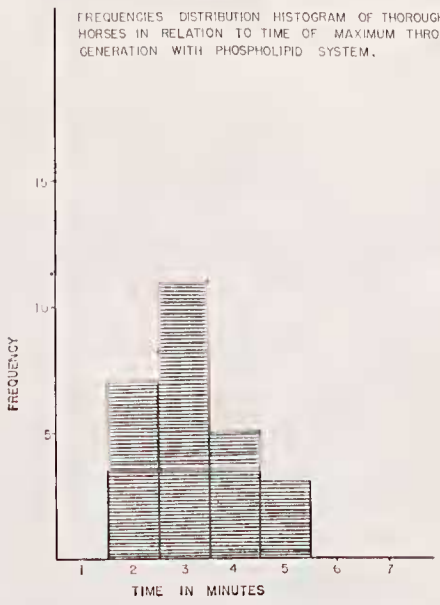


FIGURE IV

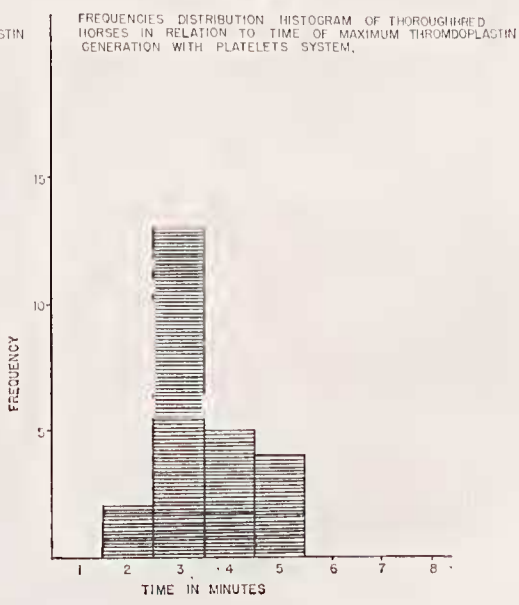


TABLE IV — SUMMARY OF VALUES IN FORMER PUBLICATIONS ON NORMAL HORSES, AND CLASSIFIED HERE ACCORDING TO THE TESTS PERFORMED BY THE AUTHORS, COMPARED TO OUR RESULTS

Tests / Authors	Clotting time (min.)	Clotting time of recalcif. plasma (sec.)	Platelets (10^3/mm^3)	Clot retraction (%)	Prothrombin time time concentraction (sec.)	Prothrombin time time concentraction (%)	Pro-thrombin consumption (%)	Thromboplastin generation: Max. gener. min.	Thromboplastin generation: Max. activ. (sec.)	Thromboplastin generation: Max. activ. concent. (%)
Adams (1)	10,47 a 16,25	—	—	—	—	—	—	—	—	—
Archer (4)	6,5-9,0 a	—	90-170 a	45-60 a	11-15 a	—	—	4-5 a	11 a	—
Araujo (3)	—	—	—	—	13	—	—	—	—	—
Awad & Morcos (5)	3,12-4,47	—	—	—	—	—	—	—	—	3-12 b
Barkhan & col. (7)	—	—	134 a	—	10,5 a 21,0 a 10,4 20,0	—	—	—	—	—
Bell & col. (12)	20-32	—	255	3-16	—	14-16 b	—	4-5	±30	—
Burker (16)	11,5 d	—	132-276	—	—	—	—	—	—	—
Buruiana & col. (18)	15-30	—	—	—	23,8	—	—	—	—	—
De Nicola & col. (22)	—	±130 e ±180	—	—	9,5 e 10,5	—	—	—	—	—

Diaz (23)	—	—	—	—	8,6 a 8,5	—	—	—	—	—
Doroschkin (24)	—	—	—	—	17,7	—	—	—	—	—
Florio & col. (28)	—	—	—	—	12,5 a 17,1	—	—	—	—	—
Hickmet (30)	—	—	350	—	—	—	—	—	—	—
Kment (34)	—	—	—	—	26,6	—	—	—	—	—
Pinkiewicz (38)	—	—	—	—	13	—	—	—	—	—
Quick (42)	—	—	—	—	—	40 % c	—	—	—	—
Rebernack (45)	—	—	250-560	—	—	—	—	—	—	—
Schwayer (50)	54 d	—	—	—	—	—	—	—	—	—
Sippel (51)	12 d	—	—	—	—	—	—	—	—	—
Sopeña (54)	—	—	305-384	—	—	—	—	—	—	—
Van Wassenhove (58)	—	—	—	—	40-50	—	—	—	—	—
Villard (59)	2-3 d	—	—	—	9-10	—	—	—	—	—
Wirth (62)	—	—	350	—	—	—	—	—	—	—
Oúr results	—	—	360	—	—	—	—	—	—	—
(means)	7,5 a	240,6 a	105 a	43,3 a	8,6 a	120,5 a	78,5 a	3,1 a 3,4 a	18 a 35,9 a	107,2 a 101,0 a

a — Thoroughbred horse b — Related to human c — Related to rabbit d — At 25°C or less e — Young animal

Discussion

Analysing the coagulation phenomenon in horses we find a series of controversial aspects both in relation to the thromboplastic factors and to the prothrombinic complex. On the other side, hemorrhagic diseases have been appointed even though the real cause for these disturbances are still unknown.

It seems to us that the greatest difficulties in the characterization of hemorrhagic syndromes in horses, are due to the fact that in the concerning literature there is not a basic work, where the different tests applied in the diagnosis of the errors of coagulation, are perfectly padronized for this kind of animal and which results can be safely used as comparative standards.

The analysis of the consulted works either aimed at the determination of averages for horses or as comparative studies between horses and other species, showed that they cannot, in many cases, be taken as reference, because they have not taken into consideration important factors for the perfect characterization of the animals, such as race, etarinm factor, moment of collection in relation to feeding, size, etc. The importance of these factors in the final results of the tests can be verified by the works of Adams (1), Awad and Morcos (5), De Nicola et al. (22), Diaz (23), Florio et al. (28), Kment (34) and Villard (59).

It can be noticed that there is an oscillation in the results when diverse techniques or variations of the same technique were used, however, even within the same technique, work conditions such as temperature, cause great errors, fact that can be easily understood if one remembers that blood coagulation is an enzimatic process in many aspects. Works by Adams (1), Burker (16), Schwayer (50), Sippel (51) and Villard (59) perfectly demonstrate this fact.

Baserga and De Nicola (12) verified that it occurs modification in blood coagulation after the injection of sexual hormones or after spontaneous modifications of the hormonal equilibrium, while Rosenfeld and Nahas (47) concluded that the prothrombinic complex does not suffer any influence either by the benzoate or the hexahydrobenzoate of estradiol or by the hexahidrobenzoate of tesosterona. De Nicola (22) found variation in number and lessening of platelet thromboplastinic activity while menstrutions took place and this has been confirmed by Introzzi and De Nicola (32). These verifieations took us to look for eventual differences caused by the sex in the results of the different test made.

Considering mentioned points such as the fact that we have been working on Thoroughbred Horses, that a definite direction has been followed in selecting the animals and applying the techniques, it seems to us that the results should be as exact as possible and this will make their indication possible, as standards for a definite population within the limits of the used pattern.

Examining the obtained results we find that in all tests the dependence intervals of 95% for the average populational values estimated for male and female horses, always show a variation zone which indicates that the differences between sex is not significant at the adopted rejection level of 5%, confirming Archer's (4) suggestion. That fact allowed us to aggregate the obtained results for male and female horses in each test, and estimate the dependence limits of 95% of the average populational values.

In relation to the authors who worked on Thoroughbred Horses, our results, for the test of blood coagulation time, are numerically inferior to Adams's (1) who used the same technique with slight modifications and coincide with Archer's (4) when analysing them in function of the value obtained for the first tube.

As for the people who worked with other races or no definite race, we mention Bell et al. (12) and Fantl and Marr (25) who got values much higher than ours using the same technique, though with few and heterogeneous samples. Burker (16) and Villard (59) respectively found higher and lower values than ours, but working at the temperature of 25°C. The results of Schwayer (50) and other authors mentioned by him, Sippel (51) and Awad and Morcos (5) cannot be compared to ours due to the different applied techniques.

The differences that we have found between the results in the first and the second test tube show that there is a technical problem. The realization of the test demands two or three test tubes, but it was never established which test tubes indicates the result; based on experimental facts, our results define that it should be the first one, because the time is shorter and the variability of the results is lower. The second test tube should be observed only as a control tube in order to avoid occasional mistakes of great range.

The test of "Coagulation time of recalcified plasma" apparently has not yet been done in Thoroughbred Horses. Results obtained by De Nicola (22) with few samples, in common old and young horses, were lower than ours, respectively in 60 and 110 seconds.

The method of Feissly and Ludin (27) has not yet been used for platelet counts in horses's blood. It is very difficult to critically analyse the results of this test which presents the higher variation according to the technique used. Just as an illustration we've noticed found that our results were a little under the ones finded by Barkhan et al. (7) and Archer (4), who made determinations in Thoroughbred Horses using methods of direct counts. Hickmet (30), Rebernack (45), Sopeña (54), Wirth (62), Wober (63) using Kocher-Fonio's technique, Behrens (9) using Neumann and Monreal's technique and Weiser (61) obtained values markedly higher than ours.

The percentage of clot retraction that we registered is slightly lower than Archer's (4) who utilized McFarlane's technique though with very few samples.

Our prothrombin times are only comparable to the ones by authors who used thromboplastin of rabbit's cerebrum (brain) for this is the most active one; even thromboplastin of horses cerebrum's acting in homologous systems, is less active as it has been demonstrated by Diaz (23). As already known, other thromboplastins are notably less active (7). The results given by us, in seconds, do not differ from the ones obtained by Diaz (23) in Thoroughbred Horses, at rest, utilizing thromboplastin of rabbit's cerebrum; the averages of 8.6 seconds are identical. We should yet consider that both samples were equivalent as to the number of animals, for that author worked with 60 animals. Barkhan et al. (7) and Archer (4) obtained higher results applying however, thromboplastin of horses's cerebrum. Comparing with results obtained in horses of different races, our prothrombin times are lower than Aranjo's (3) and Kment (34) with thromboplastin of rabbit's cerebrum, Van Wassenhove's (58) with thromboplastin of bovine's lung. Villard (59) with thromboplastin plus calcium "Biolyon", Doroschkin's (24) with human thromboplastin and Pinkiewicz (38) with thromboplastin "Roche". The percentual values are not comparable since they vary in function of the standard considered as 100%.

The results of the test of usage of prothrombin indicate that over 75% of the plasmatic prothrombin is consumed 60 minutes after the beginning of the coagulation process and we could not find literature on the subject to compare with.

The thromboplastin generation test will be considered under two aspects:

First with respect to the time necessary to happen the maximum generation. Examining the tables and graphs IV and V we verify that for male and female horses, either applying platelets suspensions of the animal itself or phospholipids of human cerebrum, the systems show maximum activity always between the second and fifth minutes. In the systems with platelets suspension, the average was 3.4 minutes and for system with phospholipids 3.1 minutes. This results acquiece with Archer's (4) verifications, who utilizing phospholipids of horses, found the maximum generation between the fourth and fifth minute. The application of phospholipids of human and not horses's cerebrum is completely explained in Barkhan et al. (7) demonstration, by which, both the lipidic fraction of human or horses cerebrum may substitute the platelets suspension as a source of platelet thromboplastinic factors, with no specificity of species.

The fact that the maximum generation always occurs between the second and the fifth minutes makes possible our advice that the test should be done within this time interval, sufficient to orientate a clinic interpretation, greatly simplifying its realization.

The second aspect to be considered is the verification of the thromboplastinic activity at the moment of its maximum generation, value which is expressed by the coagulation time of the oxalated plasm poor of platelets. One can easily realize, by analysing the results, that though the minute in which the maximum

generation occnrs, is the same for either systems with platelets or phospholipids, the thromboplastinic activity is greater in the last case, fact which has been verified in a general way by Bell and Alton (10) and might also confirmate Fantl and Marr's (25) conclusions. However, from these consideration, we cannot get conclusions also because the experiments were not done in a way to permit comparisons of the different methods. The thromboplastin activity in its maximum generation in systems with human phospholipids, appointed as average, is a little higher than the value for an animal found by Archer (4). Percentual comparison is not possible because it had to be done in function of the concentration curve which depends on the standard considered as 100%.

There was not care taken in evidenciating sex differences when results were changed into percentage, for this analysis was done though values expressed as time unity. As no meaningful differences were found, both male and female horses were used to determine the curve of thromboplastin dilution. Same points were taken into consideration for prothrombin time.

Summary

The most usual test were carried out to the evaluation of the phenomeno of blood coagulation in Thoroughbred Horses 25 males and 25 females, with ages between 2 and 5. No significant differences were found between sexes in the results of different tests at the rejection level of 5%. The average values found according to the pooled data of males and females were: coagulation time: first tube: 7.5 minutes ± 1.1; second tube: 8.9 minutes ± 1.4; coagulation time of recalcified plasma: 240.6 seconds ± 69.2; platelets: 105,000/mm³ ± 33.000; prothrombin time: a) 8.6 seconds ± 2.5; b) 120.5% ± 58.0; prothrombin consumption: 78.5 ± 13.5; thromboplastin generation: A) System with human brain extract: a) maxima generation minute: 3.1 ± 0.9; b) maxima activity in coagulation time of substract plasma: 18.0 seconds ± 6.0; c) activity of maxima concentration: 107.2% ± 20.5; B) System with platelets of the animal itself: a) maxima generation minute: 3.4 ± 0.8; b) activity in coagulation time of substract plasma: 35.9 seconds ± 10.3; c) activity of maxima concentration: 101.0% ± 31.7.

The result of blood coagulation time to be considered when the Lee and White technique is employed using two tubes, should be the one obtained with the first tube, because the time is shorter and the variability of results smaller and the second tube ought to be observed only as a witness, to avoid random errors of grate range.

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